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Ultrasound bath-assisted extraction of essential oils from clove using central composite design

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ABSTRACT

Ultrasound-assisted extraction of essential oils from clove was carried out using central composite design (CCD). The extraction parameters were optimized with response surface methodology (RSM). Three independent variables were extraction temperatures (ranging from 32 to 52 °C), extraction times (ranging from 30 to 60 min), and plant concentrations (ranging from 3 to 7%). The dependent (response) variable was clove extract. In this study, all the experiments were carried out in an ultrasound bath with a frequency of 53 kHZ. A high coefficient of correlation (0.94) was obtained between the predicted and actual clove extract yields. This result demonstrates the validity of the model used in the experiment. The statistical results showed that the extraction temperature had the most significant influence on the clove extract yield. The clove extract contained the following compounds: eugenol, α-caryophyllene, and 2-methoxy-4-(2-propenyl) phenol acetate. The major compound in the clove extract was eugenol. Antibacterial studies showed that essential oils derived from the ultrasound extraction of clove may be used as alternative bactericidal and bacteriostatic agents in the pharmaceutical industry.

1. Introduction

The extraction of essential oils from plants has been the subject of great research interest due to their potential uses in a variety of applications, including food preservatives, pharmaceutical medicines, and cosmetics. Essential oils derived from aromatic plants have many useful biological properties whereby they can exhibit antibacterial, antifungal, and antioxidant activity (Scopel et al., 2014). There are various extraction methods available (i.e., steam distillation, percolation, microwave-assisted extraction, supercritical fluid extraction and ultrasound-assisted extraction) to extract essential oils from plants. The extraction and purification of chemical compounds from plants using conventional methods have some disadvantages. In conventional methods the extraction efficiency is relatively low and higher amounts of reagents are required for the extraction (Kimbaris et al., 2006). Furthermore, relatively mild temperatures are required in the extraction of essential oils because most of the active compounds in plants are thermally unstable and may decompose during thermal extraction and distillation methods (Roldán-Gutiérrez et al., 2008). Ultrasound-assisted extraction (UAE) comes to the forefront with several advantages. The UAE method is not only a clean process, but also ultrasound effect improves the extraction efficiency by increasing the penetration of solvent into the plant cells via cavitation (Alexandru et al., 2013). Another advantage of the UAE process is that it prevents the degradation of extracts (Roldán-Gutiérrez et al., 2008).

Clove (*Syzygium aromaticum*) is one of the most important aromatic herbs, which is used as a food ingredient, a food preservative and a pharmaceutical medicine. Most of previous works regarding the extraction of essential oils from clove have been carried out using supercritical fluid technology (Clifford et al., 1999; Guan et al., 2007; Scopel et al., 2014). Supercritical fluid extraction of essential oils from clove using CO_2 has been carried out by Scopel et al. (2014). The extraction parameters tested in this study were extraction temperature (40 and 50 °C), pressure (90 and 100 bar), and extraction time (ranged from 0 to 240 min). The highest extraction yield was approximately 14.2% and it was obtained at the following conditions: 40 °C extraction time, 90 bar pressure, and 240 min extraction time. The major compounds identified in the clove extracts were eugenol and caryophyllene. In another study, supercritical carbon dioxide ($ScCO_2$) has been used to extract essential oils from clove buds at an extraction temperature of 55 °C, a

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pressure of 200 bar, and an extraction time of 330 min; likewise, superheated water has been used at an extraction temperature of 150 °C and an extraction time of 100 min (Clifford et al., 1999). This study compared the yields of the three main compounds (i.e., eugenol, eugenol acetate and caryophyllene) in mass percentage. The yields of eugenol and eugenol acetate were similar (about 16%). However, the yield of caryophyllene was lower for the superheated water extraction process. Guan et al. (2007) investigated the extraction of essential oils from clove buds using Sc-CO_2. The effects of the extraction parameters [i.e., extraction temperature (30, 40, 50 °C), pressure (10, 20 and 30 MPa), and particle size] on the extraction yields and the content of eugenol in extracts have been investigated using a three-level orthogonal array design. It was reported that the most significant factors were the extraction temperature for the eugenol content in extracts and particle size for the extract yields. The highest essential oil extract yield was 19.56% with eugenol content of 58.77%. To our knowledge, there is only one study regarding the UAE of clove buds to assess the efficiency of both batch and flow ultrasonic reactors (Alexandru et al., 2013). In that study, the experiments were conducted using clove buds of different origin (India, China and Madagascar), and three different flow rates (450, 900 and 1350 mL/min). The highest yields were obtained using a flow reactor at higher flow rates for all kind of clove buds.

In any extraction method, the extraction parameters have significant effects on the extract yields. The optimization of extraction parameters is generally carried out with a classical optimization technique in which one variable is changed at a time while the other variables are kept constant (Kannan et al., 2004). However, this technique does not provide any information on the interactions among the experimental variables tested in the experiment (Bezerra et al., 2008). Also, this classical optimization approach is time consuming and it requires a large amount of material (Ramić et al., 2015; Şahin and Şamlı, 2013). Using an experimental design to optimize experimental parameters is time saving and leads to a reduced number of experiments which results in the use of less material and reagent. Also, this method gives information about interactions among the variables (Akalın et al., 2013; Leardi, 2009; Tekin et al., 2015). In previous works, response surface methodology with different experimental designs to optimize extraction parameters for the UAE of plants has been widely investigated (Danlami et al., 2014; Majd et al., 2014; Paz et al., 2015). To the best of our knowledge, no other study has been completed regarding the application of RSM to the extract yields from clove using UAE. In this study, ultrasonic extraction parameters were optimized using response surface methodology with a central composite design for the extraction of essential oils from clove. The composition of the essential oil was also investigated as well as the antibacterial activity.

2. Experimental

2.1. Materials

Clove buds were purchased from a local market in Karabük, Turkey. Air-dried samples were ground with a blender and sieved, so that particle sizes of <0.5 mm could be chosen for the extraction studies. The sieved clove particles were thoroughly mixed and stored in plastic bags at ambient temperature, and protected from light before being subjected to the ultrasound-assisted extraction. All of the chemicals used in the experiments were analytical-grade and used without further purification. Ethanol and dimethyl sulfoxide (DMSO) were purchased from Sigma–Aldrich, USA. Mueller Hinton Agar and Mueller Hinton Broth were obtained from Merck, Germany. Chloramphenicol (C30) was supplied from Himedia Laboratories, India.

2.2. Ultrasound-assisted extraction procedure

The UAE of clove was conducted in an ultrasonic bath (KUDOS; 53 kHz frequency; power range 40–100%). The UAE experiments were carried out at different extraction temperatures, extraction times, and plant concentrations (%) according to the designated experimental design. Plant concentration (%) is defined in the equation below:

$$\text{Plant concentration}\,(\%) = \frac{\text{weight of plant}\,(\text{g})}{\text{volume of ethanol}\,(\text{mL})} \times 100$$

Clove samples and ethanol were placed into an Erlenmeyer flask, and subsequently the flask was placed into the water bath. After completion of the UAE experiments, the mixture of liquids and solids were separated using vacuum filtration. After filtration, the extracts of clove were obtained by removing the solvent in a rotary evaporator. The following equation was used to calculate the extract yields of clove:

$$\text{Clove extract yield}\,(\%) = \frac{\text{weight of clove extract}\,(\text{g})}{\text{weight of the raw material}\,(\text{g})} \times 100$$

2.3. Experimental design and statistical analysis

To optimize UAE parameters, a central composite design (CCD) with two replicates at the center points for each block was used. The three factors tested in the experiments were extraction temperature, extraction time, and plant concentration which were coded as x_1, x_2, and x_3, respectively. The response surface with a second-order polynomial model was used to correlate between responses and changeable parameters (Akalın et al., 2015; Myers et al., 2009). The mathematical model used for the three-variable CCD is given below and designated Eq. (1). The second order polynomial model, which relates response to the factors, consists of 1 intercept term, 3 squared terms, 3 two factor interaction terms and 3 linear terms:

$$Y_i = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 \quad (1)$$

where Y_i is a dependent variable (clove extract yield); β_0 is a constant; β_1, β_2, and β_3 are the linear coefficients; β_{11}, β_{22}, and β_{33} are the quadratic coefficients; β_{12}, β_{13}, and β_{23} are the interaction coefficients; and x_1, x_2, and x_3, are the coded values of the independent variables. A total of eighteen experiments including two center replicates for each block were carried out using statistical software [STATISTICA (version 8.0)]. The factorial design points were displayed in one block and the axial points were displayed in another block in standard orders.

2.4. Analysis

The selected extract of clove was analyzed by gas chromatography-mass spectrometry (GC–MS) using an Agilent 6890 gas chromatograph [30 m × 0.25 mm i.d. phenyl methyl siloxane capillary column (HP-5MS) with a film thickness of 0.25 mm]. The following temperature program was used: started at 40 °C for 10 min; 40 → 170 °C (at a heating rate of 3 °C/min for 5 min); 170 → 250 °C (at a heating rate of 4 °C/ min); 250→300 °C (at a heating rate of 12 °C/min); and held for 10 min at the final temperature. The injector temperature was 250 °C with a split mode. Helium was used as the carrier gas at a flow rate of 1 mL/min. The column was directly introduced into the ion source of an Agilent 5973 series mass selective detector operated with an